Detection of the extranuclear-to-nuclear ratio of ERα by phosphor-integrated dots and prognostic value of resistance to endocrine therapy in HR+/HER2- breast cancer

（書式１８）課程博士

学　位　論　文　要　約

博士論文題目　Detection of the extranuclear-to-nuclear ratio of ERα by phosphor-integrated dots and prognostic value of resistance to endocrine therapy in HR+/HER2- breast cancer（蛍光ナノ粒子を用いた核外/核内 ERα定量化によるホルモン受容体陽性/HER2陰性乳癌の内分泌療法耐性の予測）

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Background In addition to genomic signalling, oestrogen receptor α (ERα) is associated with cell proliferation and survival through a nongenomic (extranuclear) signalling pathway, which is considered to contribute to endocrine resistance. However, the relationship between extranuclear ERα expression levels and endocrine therapy (ET) resistance has not been studied extensively. My goal was to assess ERα expression levels in the cytoplasm and/or at the membrane in breast cancer specimens using IHC with PIDs (IHC-PIDs) as a retrospective research tool and to explore the significance of extranuclear ERα in predicting the benefit of ET and patient prognosis. **Methods** ERα expression levels were quantified in cells lines and tissue section with a range of increasing ERα expression by using IHC-PIDs, flow cytometry and real-time quantitative reverse transcription-PCR (real-time qRT-PCR). GFP-ERαΔNLS-transfected cells were stained with PIDs to detect extranuclear ERα. Then, sixty-five patients with HR+/HER2- breast cancer from 2001 to 2003 who were treated with postoperative ET were selected. Tissue sections were stained with IHC-PIDs, and total ERα, nuclear ERα, extranuclear ERα, and the ratio of extranuclear-to-nuclear ERα (ENR) were then measured with an automatic computerized measurement named “the nearest-neighbour method”. The correlations among biological factors, clinical characteristics and pathological features were examined. **Results** PIDs were able to specifically quantify ERα as the results of strong correlation between PIDs score and mRNA expression (Pearson r^2=0.94) as well as FACS (Pearson r^2=0.98). PIDs exhibit appropriate recognition of extranuclear ER in cancer cells. 4 μm was chosen as the optimal value for the nearest-neighbor method which provides an accurate measurement of the extranuclear ERα protein expression level. The PIDs scores of total and nuclear ERα negatively correlated with relapse, and the ERα ENR calculated by the nearest-neighbour method was positively correlated with relapse. A high (≥48) nuclear ERα PIDs score was associated with better disease-free survival (DFS) (log-rank P=0.022). However, a high (≥0.5) ERα ENR was significantly associated with poor OS (log-rank P=0.048) and DFS (log-rank P=0.007). Multivariate analysis revealed that the ERα ENR remained an independent prognostic factor for DFS [hazard ratio, 3.8; 95% CI, 1.4~11.8; P=0.006]. **Conclusion** I used IHC-PIDs imaging to develop a novel automatic computerized measurement with high accuracy for localizing and assessing extranuclear ERα, which was previously rarely detected. I demonstrated a new method for localizing and assessing extranuclear protein that can be applied to other biomarkers to explore their underlying mechanisms. A high ERα ENR and/or low nuclear ERα PIDs score in HR+/HER2- breast cancer indicates a decreased chance of benefitting from ET.